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Synthesis of new *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-[(substituted)thio/piperazine]acetamide/propanamide derivatives and evaluation of their AChE, BChE, and BACE-1 inhibitory activities

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Abstract

In this study, the synthesis of *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-[(substituted)thio/piperazine]acetamide/propanamide derivatives (**3a**-**3k**) and to investigate their acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and β-secretase 1 (BACE-1) inhibition activity were aimed. Mass, ^{1}H NMR, and ^{13}C NMR spectra were utilized to determine the structure of the synthesized compounds. Compounds **3b**, **3c**, **3f**, and **3j** showed AChE inhibitory activity which compound **3c** ($IC_{50} = 0.030 \pm 0.001$ μM) showed AChE inhibitory activity as high as the reference drug donepezil ($IC_{50} = 0.0201 \pm 0.0010$ μM). Conversely, none of the compounds showed BChE activity. Compounds **3c** and **3j** showed the highest BACE-1 inhibitory activity and IC_{50} value was found as 0.119 ± 0.004 μM for compound **3j** whereas IC_{50} value was 0.110 ± 0.005 μM for donepezil, which is one of the reference substance. Molecular docking studies have been carried out using the data retrieved from the server of the Protein Data Bank (PDBID: 4EY7 and 2ZJM). Using in silico approach behavior active compounds (**3c** and **3j**) and their binding modes clarified.

KEYWORDS

Alzheimer disease (AD), benzothiazole, cholinesterase inhibitors, β-secretase (BACE-1)

1 | INTRODUCTION

Dementia is a term used to describe several conditions that affect memory, thinking and the ability to carry out daily activities. A total of 55 million people worldwide have dementia. Nearly 10 million new cases are diagnosed each year. Alzheimer disease (AD) is the most common form of dementia, accounting for 60%–70% of all cases (Sang et al., 2022). AD is a progressive neurodegenerative condition characterized by age-related memory loss. It was first described by Dr. Alois Alzheimer in 1906 (Deture & Dickson, 2019). Due to the complexity of the disease, there is still a lack of understanding of the underlying mechanisms of AD (Erdogan et al., 2021).

The most elucidated pathogenesis of AD is the cholinergic deficiencies that cause the loss of memory and learning ability (Tok et al., 2022). The cholinergic hypothesis proposed that Alzheimer is caused by a lack of a key neurotransmitter called acetylcholine (ACh)

in the neuronal gaps (Chen et al., 2022; Walczak-Nowicka & Herbet, 2021). Acetylcholinesterase (AChE) is an enzyme that hydrolyzes acetylcholine to choline and acetic acid (Sağlık et al., 2020; Zhao et al., 2017). Inhibiting AChE increases acetylcholine levels by preventing its breakdown in brain tissue, thus improving cholinergic function in Alzheimer patients (Moss, 2020; Sahin et al., 2020; Srivastava et al., 2021). AChE inhibitors are the main class of drugs used to treat AD. Four out of the five drugs (tacrine, donepezil, galantamine, rivastigmine) approved by the Food and Drug Administration and available for the treatment of AD are AChE inhibitors (Marucci et al., 2021; Sağllk, 2022; Uytun et al., 2022).

An alternative approach to treating AD is to block the formation of amyloid beta (Aβ) plaques (Gabr & Abdel-Raziq, 2018; Zhao et al., 2017). The two-step proteolysis of amyloid precursor protein (APP) involves three membrane-bound enzyme complexes, α-secretase, β-secretase (BACE-1) and γ-secretase (Sathya et al., 2012; Vetrivel & Thinakaran, 2010). Together with gamma secretase, beta secretase (BACE-1) cleaves the APP to generate the different Aβ species (Evin et al., 2010; Pont et al., 2021; Qu et al., 2021). BACE-1-mediated cleavage is the rate determining step of the Aβ generation which is an attractive potential therapeutic target as it plays a key role in the pathway of Aβ formation (Ferreira et al., 2021; Reza-Zaldívar & Jacobo-Velázquez, 2023; Sharma et al., 2019). It is thought that inhibition of BACE-1 reduces the amount of Aβ produced and thus this situation slows down or halts the progression of AD (Hampel et al., 2021; Huang et al., 2010). Over the past few years, medicinal chemists have synthesized compounds that might have BACE-1 inhibitory effect, except aducanumab, which is a monoclonal antibody against Aβ, all the compounds have failed in clinical trials (Gehlot et al., 2022; Jeremic et al., 2021).

The drugs that are currently being used to treat AD are not as effective as hoped. In recent studies, researchers have focused on dual inhibition of the BACE-1 and AChE complexes to treat AD and other neurodegenerative disorders (Costanzo et al., 2016; Gabr & Abdel-Raziq, 2018; Rampa et al., 2015).

Thiazoles and benzothiazoles are important heterocyclic compounds with numerous therapeutic activities such as antimicrobial, antioxidant, anticancer, antimalarial, antiviral as well as anti-acetylcholinesterase activity (Keri et al., 2015; Niu et al., 2023; Rouf & Tanyeli, 2015; Sun et al., 2016). In especial, many studies have been reported the anti-Alzheimer activities of benzothiazole derivatives as anticholinesterase inhibitors and also BACE inhibitors (Demir Özkay et al., 2017; Xu et al., 2010).

By combining the 5,6-dimethoxy residue in the structure of the drug donepezil, which has strong anticholinesterase activity, and the 2-aminobenzothiazole structures found in riluzole and sabeluzole molecules, which are known for their *N*-methyl-D-aspartate receptor (NMDA) modulatory effects, 2-amino-5,6-methylenedioxybenzothiazole derivatives were designed and the AChE, BChE and BACE inhibitory activities of the compounds were examined. From a similar perspective, 5,6-dimethoxybenzothiazole-piperazine acetamide derivatives (Figure 1,A) were synthesized and anticholinesterase activity was determined with high potency (IC_{50}: 0.396–0.0462 μM) on AChE (Demir Özkay et al., 2016). In another study, AChE inhibition with IC_{50}: 0.023 μM was determined in the derivative containing nonsubstituted benzothiazole and dimethylaminoethyl residue (Figure 1,B) (Karaca et al., 2022). It was also determined that the benzothiazolacetamide derivative (Figure 1,C) containing 4-methylbenzyl exhibited anti-AChE activity with IC_{50}: 0.061 μM in a different study (Özkay et al., 2012). Additionally, in a

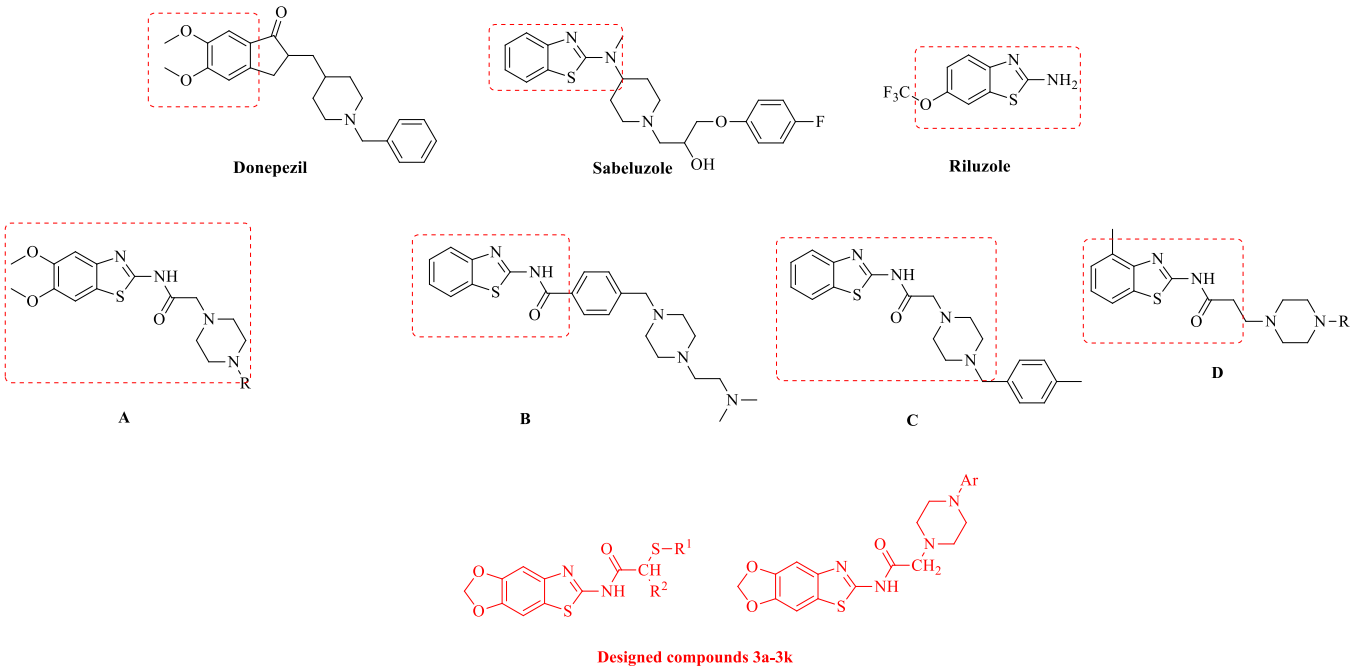


FIGURE 1 Benzothiazole framework in some studies and **3a-3k** compounds.

study conducted in 2022, a large series containing 4-methy lbenzothiazole (Figure 1,D) and cholinesterase inhibition-based multi-target anti-Alzheimer activity was investigated. The investigators suggested that four of the compounds preserve synaptic integrity and are promising in treating AD (Turgutalp et al., 2022). Based on this information, 11 novel *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-[(substituted)thio/piperazine]acetamide/propanamide derivatives (**3a-3k**) were designed synthesized evaluated for their anticholinesterase (AChE, BChE) and BACE-1 inhibitory activity, also molecular docking studies and molecular dynamic simulation studies were realized on AChE and BACE-1 enzymes for the most active compounds.

2 | RESULT AND DISCUSSION

2.1 | Chemistry

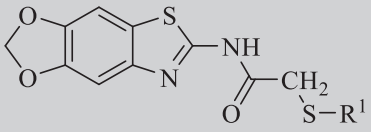
In the present study, the synthesis of novel *N*-(5,6-methylenedio xybenzothiazole-2-yl)-2-[(substituted)thio/piperazine]acetamide/propanamide derivatives (**3a-3k**) was performed in three steps starting from 3,4-(methylenedioxy)aniline. The 5,6-methylenedioxybenzothiazol-2-amine obtained in step 1 was then acetylated separately with chloroacetyl chloride and 2-chloropropionyl chloride. Finally, *N*-(5,6-methylenedio xybenzothiazol-2-yl)-2-chloroacetamide/propanamide derivatives were derivatized with appropriate mercaptoaryl or phenylpiperazine derivatives.

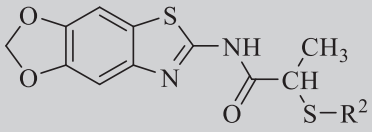
The obtained compounds were analyzed. In the supporting information, the spectra obtained are given. And the list of compounds obtained is shown in Table 1.

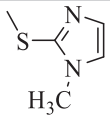
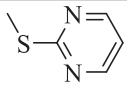
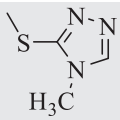
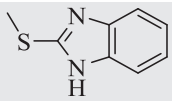
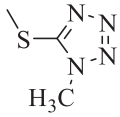
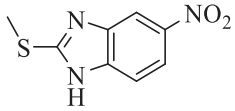
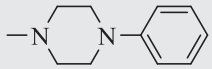
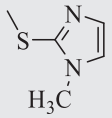
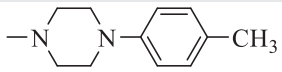
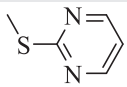
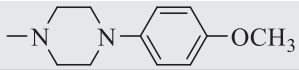
When the ^{1}H-NMR results of the final synthesized compounds were examined, it was found that the results were in the range of 1.44–12.61 ppm. In all compounds the peak belonging to the two hydrogens of the 1,3-dioxolo structure was observed in the range of 5.99–6.12 ppm. In addition, it was determined that the hydrogens bonded to carbonyl were between 3.37 and 4.40 for $COCH_2$ and between 4.15 and 4.76 for $COCHCH_3$. In addition, the peaks of the hydrogens of the benzothiazole ring were observed in the range of 7.29–7.56 ppm.

When the ^{13}C-NMR results of the final synthesis compounds were examined, it was found that the results were in the range of 17.51–171.16 ppm. In addition, the carbon atom attached to the carbonyl was found to be between 35.32 and 60.61 for $COCH_2$ and between 43.76 and 45.58 for $COCHCH_3$. A range of 100.47–101.89 ppm was observed for peaks belonging to carbon of 1,3-dioxolo structure. It was also observed that the peaks belonging to the carbons of the benzothiazole ring were found to range from 100.95 to 171.16 ppm.

TABLE 1 Synthesized compounds.





Compound	R^1	Compound	R^2
3a		3g	
3b		3h	
3c		3i	
3d		3j	
3e		3k	
3f			

Mass spectra of the synthesized result compounds were obtained using the APCI-MS method. The results showed that the molecular weights of the compounds are compatible with the M+H peak or M-H. However, the M+H peak could not be obtained because some compounds degraded during application.

2.2 | Evaluation of enzyme inhibition activity

In this study, the AChE, BChE, and BACE-1 activities of the synthesized ***N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-[(substituted)thio/piperazine]acetamide/propanamide** derivatives were investigated.

The AChE/BChE inhibitory activities of the synthesized compounds are shown in Table 2. AChE activity was observed for compounds **3b**, **3c**, **3f**, **3g**, and **3j**. Compound **3c** (IC_{50} = 0.030 ± 0.001 μM) showed the highest activity, with a value that was very close to that of the reference substance donepezil (IC_{50} = 0.0201 ± 0.0010 μM). However, no BChE activity was detected for any of the compounds.

Compounds **3b**, **3c**, **3f**, and **3j**, which were found to have the highest activity in the AChE inhibitory assay, were tested for potential BACE-1 inhibitory activity. The BACE-1 inhibitory activities of the synthesized compounds are shown in Table 3. This assay was performed using a commercially available fluorometric assay kit ("Human β-Secretase [BACE1] Inhibitor Screening Assay" kit (BioVision). Donepezil and verubecestat were used as the reference drugs in the assay. Compounds **3c** and **3j** exhibited BACE-1 inhibition activity comparable to the reference drugs. Compounds **3c** and **3j** are the two compounds that show the best inhibitory activity against AChE, as well as the two compounds that show the highest BACE-1 inhibition. The highest BACE-1 inhibitory activity was observed for compound **3j** (IC_{50} = 0.119 ± 0.004 μM). However, the BACE-1 inhibitory activity of compound **3j** is very close to that of donepezil (IC_{50} = 0.110 ± 0.005 μM), one of the reference compounds.

TABLE 2 IC_{50} (μM) values of synthesized compounds against AChE/BChE enzymes.

Compound	AChE IC_{50} (μM)	BChE IC_{50} (μM)
3a	>100	>1000
3b	0.074 ± 0.002	>100
3c	0.030 ± 0.001	>100
3d	>100	>100
3e	>100	>100
3f	0.049 ± 0.002	>1000
3g	0.190 ± 0.008	>100
3h	>1000	>1000
3i	>100	>1000
3j	0.038 ± 0.001	>100
3k	>1000	>1000
Donepezil	0.0201 ± 0.0010	–
Tacrine	–	0.0064 ± 0.0002

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase.

TABLE 3 IC_{50} (μM) values of synthesized compounds against BACE-1 enzymes.

Compounds	β-Secretase (BACE-1) IC_{50} (μM)
3b	>10
3c	0.236 ± 0.011
3f	>100
3j	0.119 ± 0.004
Donepezil	0.110 ± 0.005
Verubecestat	0.031 ± 0.001

2.3 | Results of in silico studies

2.3.1 | Molecular docking study

Docking study on AChE enzyme

To estimate the binding modes of the four active compounds (**3b**, **3c**, **3f**, and **3j**), the compounds were docked to the active site of the AChE. According to Figure 2, active compounds (**3b**, **3c**, **3f**, and **3j**), binding forms and types on AChE are listed in Table 4. The 4EY7 crystal form of the enzyme has been chosen for the docking of the compounds. According to the data provided, compound **3b** interacted with Trp286, Tyr124, Tyr341, and Trp86 via π–π stacking, with Phe295, Arg296 and His447 via H-bonds. On the other hand, compound **3c** interacted with Trp286, Tyr124, Tyr341, and Trp86 via π–π stacking, with Phe295, Arg296, and Asp74 via H-bonds. Compound **3f** only interacted with Trp286 and Try124 via π–π stacking, with Phe295 via H-bond. Compound **3j** interacted with Trp286, Tyr124, Tyr341, and Trp86 via π–π stacking, with Phe295 and Asp74 via H-bonds. Interactions with CAS (Trp86) and PAS (Trp286) amino acids are described as pivotal residues in previous study (AL-Sharabi et al., 2023). All active compounds showed affinity to these residues with at least one interaction. Commonly, active compounds interacted via π–π stacking. Only compound **3f** did not show affinity to Trp86, but still this compound occupied the CAS region. All compounds formed H-bonds with Phe295 while compounds **3b** and **3c** also formed H-bonds with Arg296. For more information, compound **3c** was used as a model for its analogs to investigate.

Docking study on BACE-1 enzyme

Molecular docking study against the BACE-1 inhibitor was performed for compound **3j**, which showed the highest BACE-1 inhibitory activity among the final synthesized compounds. According to Figure 3, the highest active compounds (**3j**), binding forms and types on BACE-1 are listed in Table 5. The 2ZJM crystal form of the enzyme was selected for docking. According to the information obtained, it was observed that

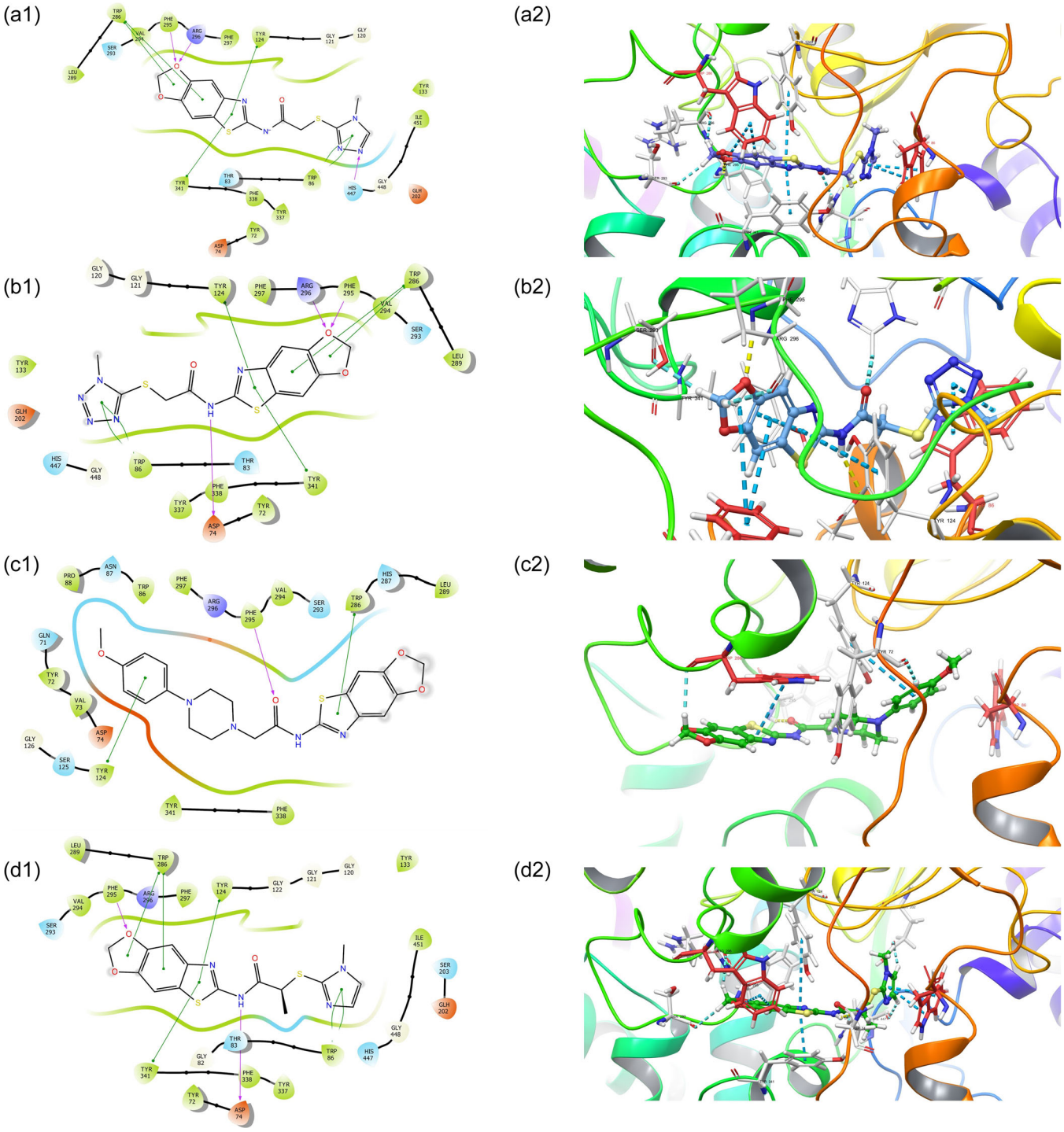


FIGURE 2 2D and 3D molecular docking poses of (a) **3b**, (b) **3c**, (c) **3f**, (d) **3j**.

compound **3j** formed hydrogen bonds with Asp32 and Thr72. It was also observed that π–π stacking occurs with Trp115 and Tyr198. All these interactions indicate that compound **3j** localized into catalytical cleft of ß-secretase enzyme. Especially, interaction with Asp32 and Thr72 residues at the same time, it's an indication that the **3j**-BACE-1 protein complex was stabilized. However, to observe this theory, the molecular dynamics simulation (MDS) study was run. The environmental changes through 50 ns were evaluated similarly as in the previous study (Hernández-Rodríguez et al., 2016).

2.3.2 | Molecular dynamics simulation

MDS of AChE

MDS study was performed on compound **3c**, which showed the highest AChE inhibitory activity among the final synthesized compounds. MDS method, apoproteins, ligand-enzyme or ligand-receptor, etc., it is a useful technique for estimating the time-dependent stability of systems (Yücel, 2024). As described previously (Dawbaa et al., 2023; Evren, 2022a; Yücel, 2024) the stability of the

TABLE 4 Summary of the interaction of 3b, 3c, 3f and 3j with the active pocket of the AChE enzyme.

Compound	Residue	Interaction number and type	Compound	Residue	Interaction number and type
3b	Trp286Tyr124Tyr341Trp86Phe295Arg296His447	1, π–π stacking1, π–π stacking1, π–π stacking1, H-bond1, H-bond	**3f**	Trp286Tyr124Phe295	1, π–π stacking1, π–π stacking1, H-bond
3c	Trp286Tyr124Tyr341Trp86Phe295Arg296Asp74	1, π–π stacking1, π–π stacking1, H-bond1, H-bond	**3j**	Trp286Tyr124Tyr341Trp86Phe295Asp74	1, π–π stacking1, π–π stacking1, π–π stacking1, H-bond1, H-bond

Abbreviation: AChE, acetylcholinesterase.

complex was protected during the entire simulation according to Figure 4a–c.

According to plots in Figure 4d–f, and the video it was observed that compound **3c** formed aromatic hydrogen bonds with Asp74, Tyr124, Trp286, His287, Glu292, Ser293, Phe295, Arg296, Phe297, Phe338, Tyr341, and Gly342. Compound **3c** contacted via water-mediated hydrogen bond with Phe295 (34%), hydrogen bond with Tyr124 (30%) and π–π stacking with Tyr341 (27%) and Trp286 (33%).

Compound **3c** has a number of important binding modes with different amino acids. Compound **3c** interacted with Trp86 (hydrophobic interaction and water-mediated hydrogen bond), Tyr124 (hydrogen bond, hydrophobic interaction, water-mediated hydrogen bond), Trp286 (hydrophobic interaction, water-mediated hydrogen bond), Phe295 (water-mediated hydrogen bond), Tyr337 (hydrogen interaction, hydrophobic interaction, water-mediated hydrogen bond), Phe338 (hydrophobic interaction, water-mediated hydrogen bond), and Tyr341 (hydrophobic interaction, water-mediated hydrogen bond).

The interaction of compound **3c** with Tyr341 was continuous and appeared to be largely hydrophobic bond and water-mediated hydrogen bonding. While Gly122 and Trp86 did not interact with compound **3c**, Tyr124 did interact with compound **3c**. However, most of the interaction of Tyr124 is via hydrogen bonding. When compound **3c** interacts less with Tyr337, it interacts more with Asp74. Water-mediated hydrogen bonding is responsible for all the interaction with Asp74. When the interaction of Phe338 with compound **3c** decreased, the interaction of Tyr337 increased. A decrease in the interaction of compound **3c** with Ser203 was accompanied by an increase in the interaction of Trp286.

MDS of BACE-1

MDS study was performed on compound **3j**, which showed the highest BACE-1 inhibitory activity among the final synthesized compounds. As described previously (in MDS results of AChE section), the stability of the complex was protected during the entire simulation according to Figure 5a–c.

According to plots in Figure 5d–f, and the video, it was observed that compound **3j** formed aromatic hydrogen bonds with Gln73, Phe108, Asp32, Thr231, Lys107, Ile118, Arg128, Ser328, and Asp228. Compound **3j** was also found to show water-mediated hydrogen bond with Asp32 (%71), Lys107 (%30), Thr72 (%43), hydrogen bond with Thr72 (%74) and π–π stacking with Tyr71 (%63–%29).

Compound **3j** has several important modes of binding with a variety of different amino acids. Compound **3j** interacted with Asp32 (water-mediated hydrogen bond), Tyr71 (hydrophobic interaction, water-mediated hydrogen bond), Thr72 (hydrogen bond, water-mediated hydrogen bond), Phe108 (water-mediated hydrogen bond).

Compound **3j** interacted continuously with Thr72 and Tyr71. The interactions appeared to be mainly via hydrophobic and hydrogen bonds mediated by water. Compound **3j** did not show any interaction with Val31, Val67, Try68, Val69, Pro70, Gly74, Trp76, and Pro129.

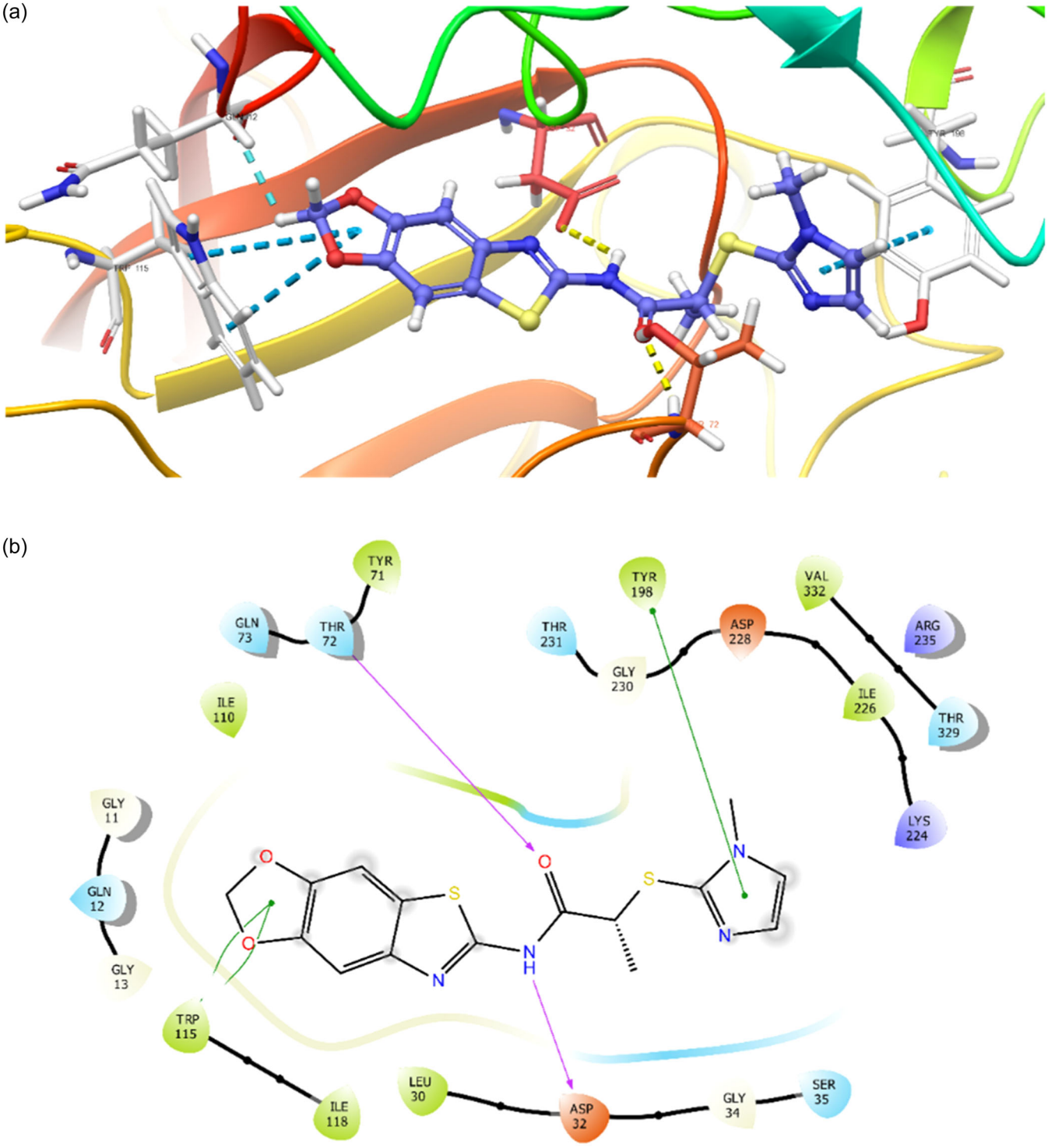


FIGURE 3 2D and 3D molecular docking poses of **3j**.

Compound **3j** has a lower interaction with Gly230 and a higher interaction with Asp32. Interaction of compound **3j** with Asp32 via a hydrogen bond mediated by water. Interactions with residues of Phe108 and Lys107 are also frequently observed. These interactions also take place via hydrogen bonds mediated by water. All these interactions are similar to docking study. The mentioned theory was tested during the simulation, and the results indicated that the most stable interactions were with Asp32 and Tyr71. Meanwhile, imidazole moiety of **3j** occupied S3 and S4 pockets of BACE-1 when 5,6-methylendioxybenzothiazole moiety docked to S2' and S3' subpockets. In conclusion, the stability of the complex made by compound **3j** and the catalytic cleft of inactive BACE-1 protein was protected during the simulation, hence, the inhibitory activity was based on the above interactions.

TABLE 5 Summary of the interaction of 3j with the active pocket of the BACE-1 enzyme.

Compound	Residue	Interaction number and type
3j	Asp32	1, H-bond
	Thr72	1, H-bond
	Trp115	2, π–π stacking
	Tyr198	1, π–π stacking

2.4 | Structure-activity relationship evaluation

The results we have obtained in our activity assays and in our molecular docking studies have shown that the mercaptoaryl or phenylpiperazine ring substituted benzothiazole ring system has AChE inhibitor activity as well as BACE-1 inhibitor activity. Most of the AChE inhibitory activity was found in compounds with a mercaptoaryl substituted benzothiazole ring system. In particular, compound **3c**, characterized by 1-methyl-2-

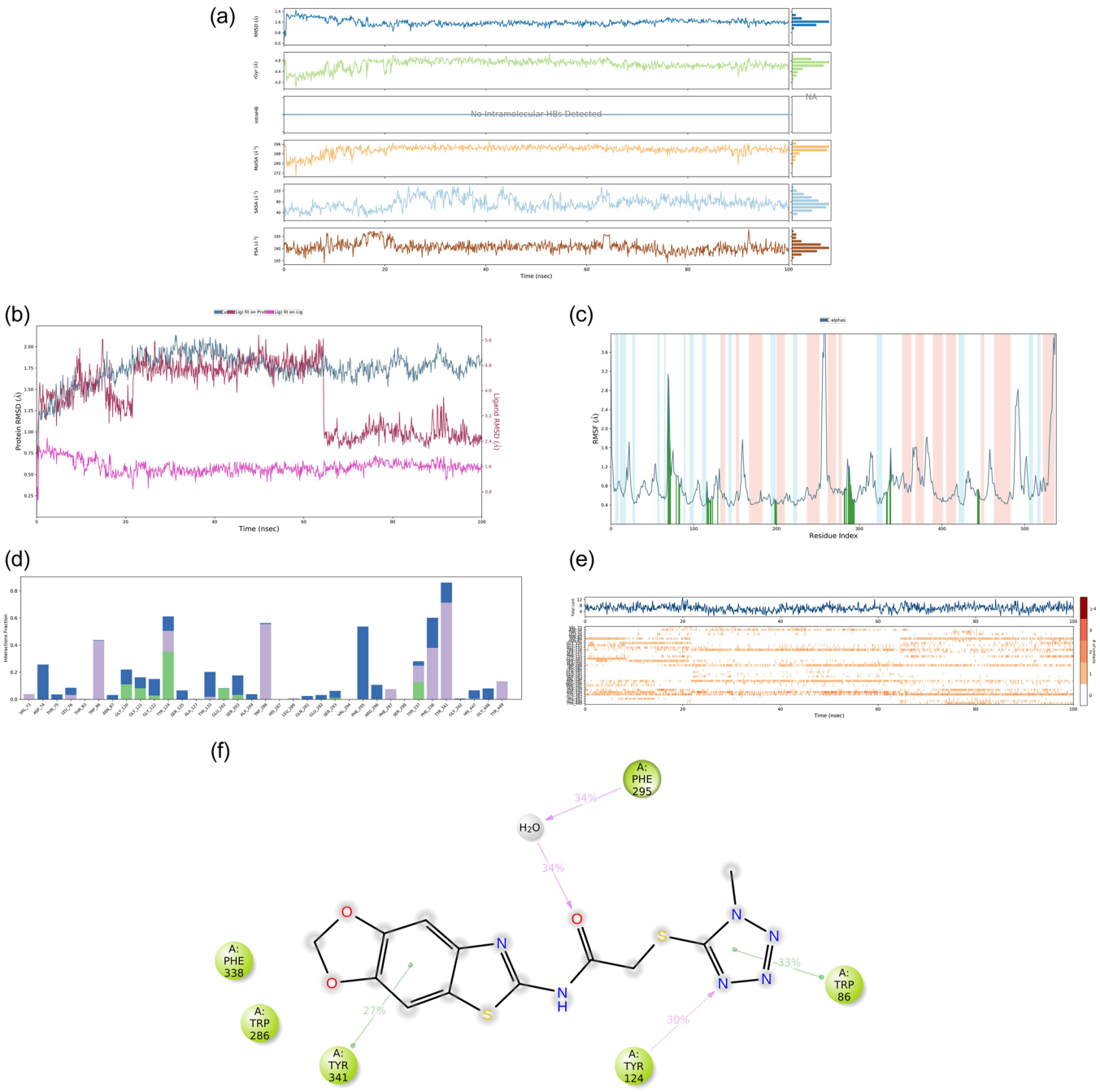


FIGURE 4 Interaction plots of the MDS results for the enzyme complexes of **3c**-acetylcholinesterase. (a) Diagram of the ligand properties, (b) RMSD plot of the ligand and of the protein throughout the simulation, (c) RMSF-plots of amino acids and their interactions, (d) Interaction between fraction and residue diagram, (e) Plot of the number of interactions—interaction types—time, and (f) Plot of the total interaction-residues-time.

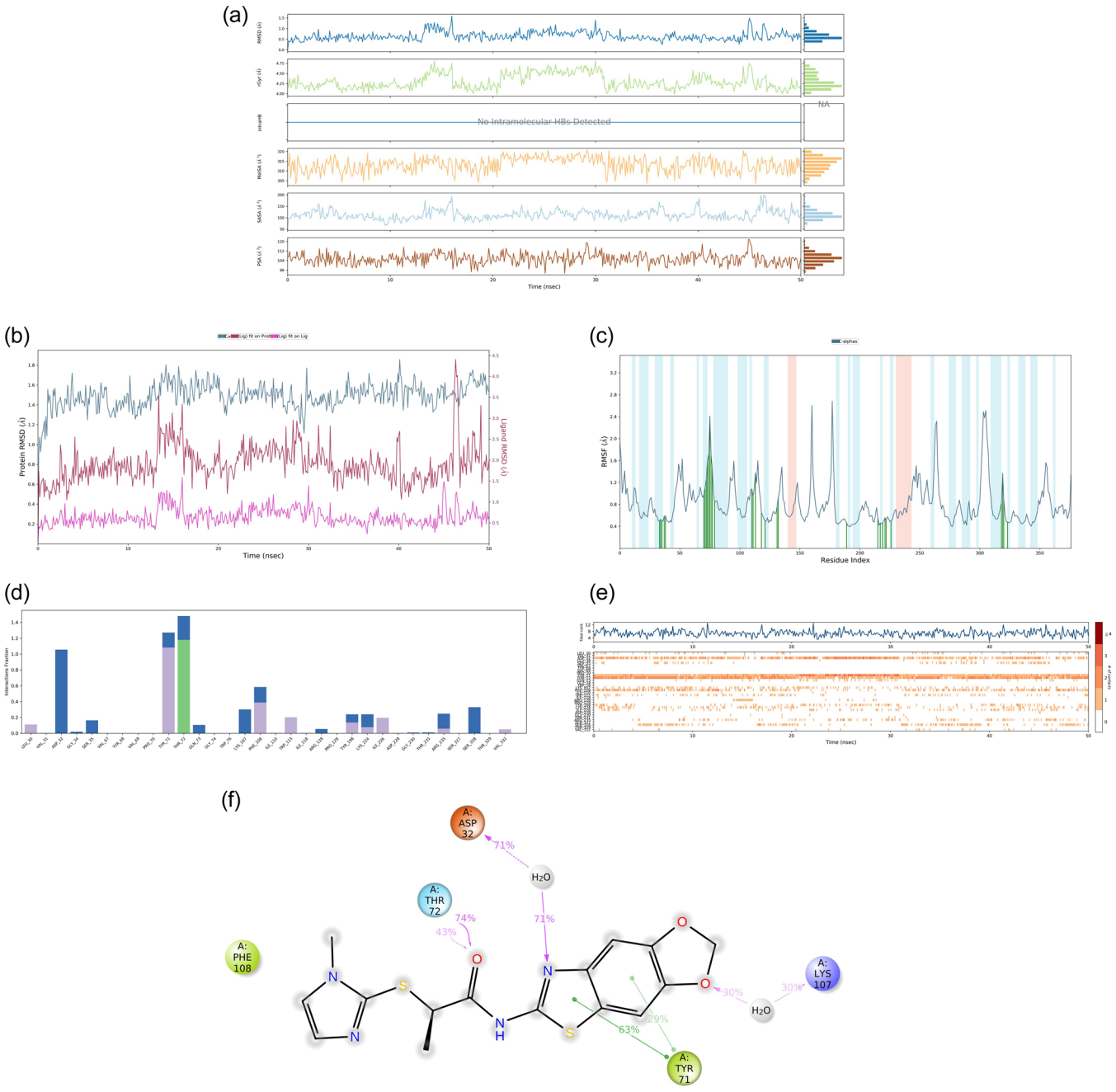


FIGURE 5 Interaction plots of the MDS results for the enzyme complexes of **3j**-BACE-1. (a) Diagram of the ligand properties, (b) RMSD plot of the ligand and of the protein throughout the simulation, (c) RMSF-plots of amino acids and their interactions, (d) Interaction between fraction and residue diagram, (e) Plot of the number of interactions—interaction types—time, and (f) Plot of the total interaction-residues-time.

mercaptotetrazole substitution, showed remarkable AChE inhibitory activity. In addition, compound **3c** has significant inhibitory activity against the enzyme BACE-1.

Significant AChE inhibitory activity was observed with the 4-methyl-2-mercaptotriazole substituted compound **3b**. On the other hand, changing the substitution to a ring with less nitrogen (e.g., compounds **3c** and **3b**) resulted in a slight decrease in AChE activity.

Among all the phenylpiperazine substituted compounds that have been synthesized, only the 4-methoxyphenylpiperazine substituted derivative, compounds **3f** showed AChE inhibitory activity.

The addition of a methyl group to the acetamide/propanamide linker group, increases the AChE inhibitory activity, and the synthesized compound can also interact with the BACE-1 enzyme and inhibit the enzyme.

In the activity test results of the AChE and BACE-1 enzyme, compound **3c** (1-methyl-2-mercaptotetrazole substituted derivative) and **3j** (1-methyl-2-mercaptoimidazole substituted derivative), showed a significant AChE and BACE-1 inhibitory activity.

TABLE 6 Physicochemical properties of compounds.

	MW	HBA	HBD	TPSA	LogP	GIA	Log K_p	DL
3a	348.80	5	1	131.81	2.08	High	−6.75	4/5
3b	349.39	6	1	144.70	1.61	Low	−7.17	3/5
3c	350.38	7	1	157.59	1.49	Low	−7.07	2/5
3d	396.46	5	1	95.17	2.68	High	−6.33	5/5
3e	410.49	5	1	95.17	2.88	High	−6.16	5/5
3f	426.49	6	1	104.40	2.60	High	−6.54	5/5
3g	346.38	6	1	139.77	2.14	Low	−6.60	4/5
3h	38.43	5	2	142.67	3.12	Low	−5.92	3/5
3i	429.43	7	2	188.49	2.22	Low	−6.31	2/5
3j	362.43	5	1	131.81	2.41	High	−6.54	4/5
3k	360.41	6	1	139.77	2.49	Low	−6.40	4/5

Abbreviations: DL, druglikeness; GIA, gastrointestinal absorption; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; log Kp, skin absorption coefficient (cm/sn); logP, partition coefficient; MW, molecular weight; TPSA, topological polar surface area (Å2).

2.5 | Results of ADME parameters and Lipinski's rule of five

The pharmacokinetic profiles of the synthesized compounds were calculated with the Swiss-ADME web-based program and whether they violated Lipinski's rule of five is shown in Table 6. The pre-calculation of ADME during drug discovery has been shown to significantly reduce the rate of pharmacokinetic-related drug failures at any clinical stage (Daina et al., 2017). The conditions required for compounds to be considered suitable for oral bioavailability are as follows MW ≤500 Da, logP <5, nHBD ≤5, nHBA ≤10, and TPSA <140 Å2 (Ya'u Ibrahim et al., 2020).

When the results were examined, it was found that the conditions of MW ≤500 Da, logP <5, nHBD ≤5, nHBA ≤10 were met for all compounds. In addition, TPSA was found to be in the range of 95.17–188.49 Å2. Furthermore, all compounds except compounds **3b**, **3c**, **3h**, and **3i** were found to have suitable TPSA. The log Kp value was found to be between −5.92 and −7.17.

3 | CONCLUSION

In this study, novel *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-[(substituted)thio/piperazine]acetamide/propanamide derivatives were synthesized in three steps starting from 3,4-(methylenedioxy)aniline. The structures of the synthesized compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. The final synthesized compounds were tested for AChE, BChE and BACE-1 enzyme inhibition.

Compounds **3b, 3c, 3f, 3g**, and **3j** showed high AChE inhibitory activity. Compound **3c** (IC_{50} = 0.030 ± 0.00 μM) showed the highest AChE inhibitory activity. Compound **3c** and **3j** showed high BACE-1 inhibitory activity. Compound **3j** (IC_{50} = 0.119 ± 0.004 μM) showed the highest BACE-1 inhibitory activity. In addition, it was determined that compounds **3c** and **3j**, which were among the compounds synthesized in this study, have both AChE and BACE-1 inhibitory activities. The results of molecular docking and molecular dynamics studies indicated that compound **3c** was constantly interacting with the active site of the AChE enzyme. The binding of compound **3j** ensured that the compound-BACE-1 enzyme complex remained stable throughout the simulation.

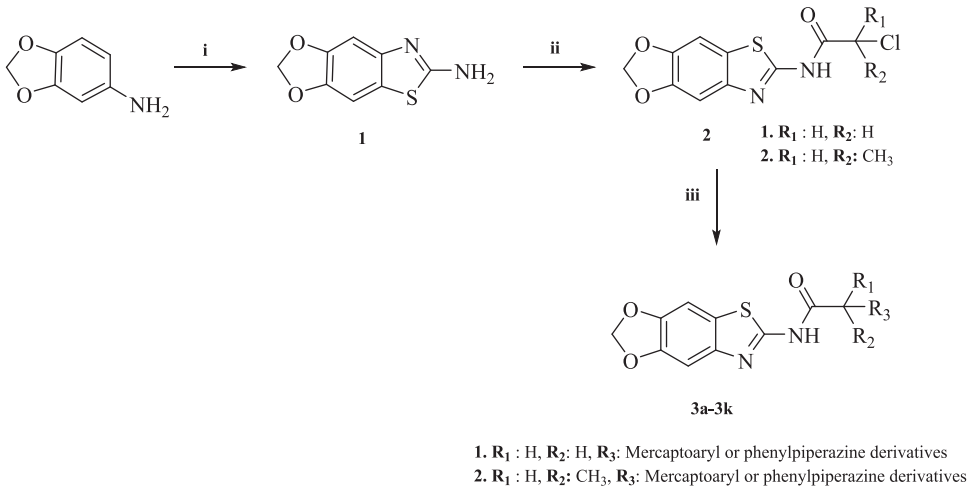
The majority of the substances that showed AChE inhibitory activity were found to be compounds with a mercaptoaryl substituted benzothiazole ring system. In particular, compound **3c**, which is characterized by the 1-methyl-2-mercaptotetrazol substitution, exhibited notable AChE inhibitory activity. Further, compound **3c** has significant inhibitory activity against the enzyme BACE-1. Compound **3b** substituted with 4-methyl-2-mercaptotriazole showed significant AChE inhibitory activity. In contrast, replacement of the substituent by a ring containing less nitrogen (e.g., compounds **3c** and **3b**) resulted in a slight decrease in AChE activity.

Several previous studies have shown that benzothiazole derivatives have a high level of cholinesterase inhibitory activity. Based on the comprehensive information available, our study showed that the newly designed benzothiazoles exhibited inhibitory effects on both the AChE and BACE-1 enzymes. In the light of this information, we aim to synthesize a new AChE and BACE-1 inhibitor based on this structure in our future studies.

4 | MATERIALS AND METHODS

4.1 | Chemistry

The chemicals used were supplied by Sigma-Aldrich Chemical (Sigma-Aldrich Corporation) and Merck Chemicals (Merck KGaA). All reactions were completed by thin layer chromatography using a silica gel 60 F_{254} pre-coated aluminum TLC plate (Merck). The melting



SCHEME 1 General synthesis scheme of the compounds. Reactions and conditions: i: KSCN, Br_2, CH_3COOH; ii: $ClCH_2COCl$ or 2-chloropropioyl chloride, TEA, THF, 0–10°C, room temperature after dropping; iii: Mercaptoaryl or phenylpiperazine derivatives, K_2CO_3, CH_3COCH_3, room temperature.

points of the final compounds were determined using the Mettler Toledo-MP90 melting point system. Nuclear magnetic resonance (NMR) spectra of the synthesized compounds were determined using Bruker DPX-300 FT NMR spectrometer and Bruker DPX 75 MHz spectrometer NMR (Bruker, UltraShield 300 MHz) spectrophotometer device. HRMS spectra were determined using Advion Compact Mass Spectrometer.

The synthesis of the *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-[(substituted)thio/piperazine]acetamide/propanamide derivatives is shown in Scheme 1.

4.1.1 | Synthesis of 5,6-methylenedioxybenzothiazole-2-amine (**1**)

Potassium thiocyanate (6.98 g, 0.072 mol) was added to 3,4-(methylenedioxy)aniline (1.92 g, 0.014 mol) in acetic acid (150 mL). This reaction mixture was placed in an ice bath and cooled to 0–10°C. Bromine (0.82 mL, 0.016 mol) in 10 mL of acetic acid was then added dropwise to the reaction mixture. After the bromine addition was completed, the mixture was stirred at room temperature for a whole day. The precipitate was then collected and boiled in water for 8 h. The resulting mixture was neutralized with ammonia solution in ice water. The resulting solids were washed with water and then dried (Karaca et al., 2022).

4.1.2 | Synthesis of *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-chloroacetamide/propanamide (2a-b)

The synthesized 5,6-methylenedioxybenzothiazole-2-amine (0.010 mol, 1.94 g) was dissolved in tetrahydrofuran (25 mL) and then triethylamine (0.012 mol, 1.7 mL) was added. This reaction mixture was placed in an ice bath and cooled to 0–10°C. Chloroacetyl chloride (0.012 mol, 0.95 mL) in 10 mL tetrahydrofuran was then added dropwise to the reaction mixture. The end of the reaction was checked by TLC after 2 h of addition. After the solvent evaporated completely, the mixture was washed with water. This reaction was also carried out using 2-chloropropionyl chloride under the same conditions (Yurttaş et al., 2014).

4.1.3 | Synthesis of *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-[(substituted) thio/piperazine] acetamide /propanamide derivatives (**3a-3k**)

A total of 0.3 g of the corresponding *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-chloroacetamide/propanamide derivatives were taken in acetone. Equal moles of the appropriate mercapto or phenylpiperazine derivatives were then added. Then, equal mole of potassium carbonate was added and stirred at room temperature. The end of the reaction was checked by TLC. After the complete evaporation of the solvent, the solid was washed with water, and then recrystallized from ethanol (Yurttaş et al., 2014).

4.1.4 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-((1-methyl-1H-imidazol-2-yl)thio)acetamide (3a)

Yield: 71% M.p. 190°C. ^{1}H-NMR (300 MHz, dimethyl sulfoxide [DMSO]-d_6, ppm) δ: 3.59 (3H, s, N-CH_3), 3.98 (2H, s, $COCH_2$), 6.06 (2H, s, O-CH_2-O), 6.95 (1H, d, *J*: 1.20 Hz, imidazole C_4-H), 7.26 (1H, d, *J*: 1.26 Hz, imidazole C_5-H), 7.29 (1H, s, benzothiazole C_7-H), 7.51 (1H, s, benzothiazole C_4-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 33.45, 37.46, 101.20, 101.34, 101.90, 124.18, 124.40, 129.07, 139.64, 143.60, 145.38, 147.74, 156.92, 167.90.

APCI-MS (-*m/z*): [M-H] $^{+}$: $C_{14}H_{12}N_4O_3S_2$ 50.

4.1.5 | *N*-(5,6-methylenedioxybenzothiazole-2-yl) calculated: 348.40, found: 348.-2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)acetamide (3b)

Yield: 68% M.p. 257°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 3.60 (3H, s, N-CH_3), 4.17 (2H, s, $COCH_2$), 6.08 (2H, s, O-CH_2-O), 7.30 (1H, s, benzothiazole C_7-H), 7.52 (1H, s, benzothiazole C_4-H), 8.57 (1H, s, triazole C_5-H), 12.51 (1H, s, N-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 31.30, 36.76, 101.21, 101.36, 101.91, 124.38, 143.67, 145.43, 146.82, 147.76, 148.85, 156.86, 167.18.

APCI-MS (-*m/z*): [M-H] $^{+}$: $C_{14}H_{12}N_4O_3S_2$ calculated: 348.40, found: 348.40.

4.1.6 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-((1-methyl-1H-tetrazol-5-yl)thio)acetamide (3c)

Yield: 73% M.p. 265°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.00 (3H, s, N-CH_3), 4.40 (2H, s, $COCH_2$), 6.08 (2H, s, O-CH_2-O), 7.32 (1H, s, benzothiazole C_7-H), 7.53 (1H, s, benzothiazole C_4-H), 12.60 (1H, s, N-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 34.18, 36.75, 101.24, 101.40, 101.94, 124.36, 143.66, 145.46, 147.78, 153.59, 156.81, 166.45.

APCI-MS (-*m/z*): [M+H]$^{+}$: $C_{12}H_{10}N_6O_3S_2$ calculated: 350.37, found: 350.5.

4.1.7 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-(4-phenylpiperazin-1-yl)acetamide (3d)

Yield: 78%, M.p. 238°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.69 (4H, t, *J*: 4.43 Hz, Ar-H), 3.17 (4H, t, *J*: 4.77 Hz, Ar-H), 3.39 (2H, s, $COCH_2$), 6.08 (2H, s, O-CH_2-O), 6.77 (1H, t, *J*: 7.15 Hz, Ar-H), 6.94 (2H, d, *J*: 8.30 Hz, Ar-H), 7.21 (2H, t, *J*:8.03 Hz, Ar-H), 7.29 (1H, s, benzothiazole C_7-H), 7.53 (1H, s, benzothiazole C_4-H), 11.98 (1H, s, N-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 48.66, 52.97, 60.58, 101.89, 115.92, 145.35, 147.71, 151.46, 156.72.

APCI-MS (-*m/z*): [M+H] $^{+}$: $C_{20}H_{20}N_4O_3S$ calculated: 396.47, found: 397.6.

4.1.8 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-(4-(p-tolyl)piperazin-1-yl)acetamide (3e)

Yield: 82% M.p. 251°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.20 (3H, s, Ar-CH_3), 2.67 (4H, brs, pip-CH_2), 3.11 (4H, brs, pip-CH_2), 3.37 (2H, s, $COCH_2$), 6.07 (2H, s, O-CH_2-O), 6.83 (2H, d, *J*: 8.06 Hz, Ar-H), 7.02 (2H, d, *J*: 7.78 Hz, Ar-H), 7.28 (1H, s, benzothiazole C_7-H), 7.53 (1H, s, benzothiazole C_4-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 20.50, 49.15, 53.00, 60.61, 101.25, 101.89, 116.19, 124.36, 128.11, 129.82, 143.64, 145.35, 147.71, 149.40, 156.73, 169.33.

APCI-MS (-*m/z*): [M+H] $^{+}$: $C_{21}H_{22}N_4O_3S$ calculated: 410.49, found: 411.6.

4.1.9 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-(4-(4-methoxyphenyl)piperazin-1-yl)acetamide (3f)

Yield: 79% M.p. 228°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.69 (4H, brs, pip-CH_2), 3.05 (4H, brs, pip-CH_2), 3.38 (2H, s, $COCH_2$), 3.68 (3H, s, Ar-O-CH_3), 6.08 (2H, s, O-CH_2-O), 6.82 (2H, d, *J*: 8.89 Hz, Ar-H), 6.89 (2H, d, *J*: 8.89 Hz, Ar-H), 7.29 (1H, s, benzothiazole C_7-H), 7.53 (1H, s, benzothiazole C_4-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 50.07, 53.09, 55.63, 101.25, 101.89, 114.71, 117.91, 124.36, 143.65, 145.34, 145.85, 147.71, 153.38, 169.34.

APCI-MS (-*m/z*): [M+H] $^{+}$: $C_{21}H_{22}N_4O_4S$ calculated: 426.49, found: 427.6.

4.1.10 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-((pyrimidin-2-yl)thio)acetamide (3g)

Yield: 84% M.p. 230°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.29 (2H, s, $COCH_2$), 6.12 (2H, s, O-CH_2-O), 7.28 (1H, m, pyridine C_5-H), 7.35 (1H, s, benzothiazole C_7-H), 7.56 (1H, s, benzothiazole C_4-H), 8.68 (2H, d, *J*:4.70 Hz, pyridine $C_{4,6}$-H), 12.61 (1H, s, N-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 34.80, 101.25, 101.88, 118.0, 124.35, 143.73, 145.33, 147.71, 157.11, 158.32, 167.60, 170.43.

APCI-MS (-*m/z*): [M+H] $^{+}$: $C_{14}H_{10}N_4O_3S_2$ calculated: 346.38, found: 346.5.

4.1.11 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-((1H-benzimidazol-2-yl)thio)acetamide (3h)

Yield: 75% M.p. Decomposed. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.06 (2H, s, $COCH_2$), 6.00 (2H, s, O-CH_2-O), 7.09-7.11 (2H, m, benzimidazole $C_{5,6}$-H), 7.13 (1H, s, benzothiazole C_7-H), 7.33 (1H, s, benzothiazole C_4-H), 7.45 (2H, m, benzimidazole $C_{4,7}$-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 38.09, 100.47, 100.95, 101.21, 114.34, 121.45, 124.95, 140.42, 143.81, 144.80, 146.68, 164.25, 171.16.

APCI-MS (-*m/z*): [M+H] $^{+}$: $C_{17}H_{12}N_4O_3S_2$ calculated: 384.43, found: not determined.

4.1.12 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-((5-nitro-1H-benzo[d]imidazol-2-yl)thio) acetamide (3i)

Yield: 80% M.p. 190°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.02 (2H, s, $COCH_2$), 5.99 (2H, s, O-CH_2-O), 7.22 (1H, s, benzothiazole C_4-H), 7.44 (2H, m, *J*: 6.01 Hz, benzothiazole C_7-H and benzimidazole C_7-H), 7.90 (1H, d, *J*: 9.02 Hz, benzimidazole C_6-H), 8.18 (1H, s, benzimidazole C_4-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 35.32, 101.28, 101.86, 110.77, 113.98, 116.74, 124.42, 141.30, 142.33, 143.77, 145.32, 147.69, 159.60, 168.14.

APCI-MS (-*m*/*z*): $[M+H]^+$: $C_{17}H_{11}N_5O_5S_2$ calculated: 429.42, found: not determined.

4.1.13 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-((1-methyl-1H-imidazol-2-yl)thio)propanamide (3j)

Yield: 71% M.p. 186°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.44 (3H, d, *J*: 7.59 Hz, COCHC<u>H</u>3), 3.58 (3H, s, Ar-H), 4.15 (1H, q, COC<u>H</u>CH3), 6.08 (2H, s, O-CH_2-O), 7.03 (1H, s, imidazole C_4-H), 7.29 (1H, s, imidazole C_5-H), 7.33 (1H, s, benzothiazole C_4-H), 7.52 (1H, s, benzothiazole C_7-H), 12.52, (1H, s, N-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 17.51, 33.68, 45.58, 101.21, 101.30, 101.45, 101.90, 124.48, 124.82, 129.53, 137.71, 143.75, 145.37, 147.73, 157.04, 170.89.

APCI-MS (-*m*/*z*): $[M+H]^+$: $C_{15}H_{14}N_4O_3S_2$ calculated: 362.42, found: 363.5.

4.1.14 | *N*-(5,6-methylenedioxybenzothiazole-2-yl)-2-(pyrimidin-2-ylthio)propanamide (3k)

Yield: 67% M.p. 160°C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.59 (3H, d, *J*: 7.02 Hz, COCHC<u>H</u>$_3$), 4.76 (1H, q, *J*: 7.11 Hz, COC<u>H</u>CH$_3$), 6.07 (2H, s, O-CH_2-O), 7.24 (1H, t, *J*: 4.92 Hz pyrimidine C_5-H), 7.32 (1H, s, benzothiazole C_4-H), 7.52 (1H, s, benzothiazole C_7-H), 8.64 (2H, d, *J*: 5.07 Hz, pyrimidine $C_{4,6}$-H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ:18.14, 31.36, 43.74, 100.27, 101.26, 101.90, 118.15, 124.46, 145.37, 147.73, 158.42, 170.28.

APCI-MS (-*m*/*z*): $[M+H]^+$: $C_{15}H_{12}N_4O_3S_2$ calculated: 360.41, found: 360.9.

4.2 | Biochemistry

4.2.1 | AChE and BChE enzyme inhibition activities

The modified Ellman method was used to determine the anticholinesterase activity of the final synthesized compounds on AChE and BChE enzymes (Ellman et al., 1961). Solutions of AChE or BChE (20 µL) prepared in 2% DMSO in the concentration range 10-1-10-6 mM and solutions of compounds (20 µL) were added to 140 µL phosphate buffer (pH 8 ± 0.1) and incubated at 25°C for 5 min. The reaction was initiated by the addition of 5-5-dithiobis(2-nitrobenzoic acid (DTNB) (20 µL) and acetylthiocholine iodide (ATC) (10 µL) to the mixture of enzyme and inhibitor. All solutions for this enzyme assay were stored at −20°C and were brought to room temperature just before the start of the experiments. After the solutions were mixed in the wells, they were incubated in an oven for 15 min at an absorbance value of 412 nm. The tests were carried out at a concentration of 10^{-3} and 10^{-4} M and the experiments were repeated four times. Lower concentrations were also tested for the determination of the IC_{50} dose for some compounds.

4.2.2 | BACE-1 enzyme inhibition activities

The experimental procedure for BACE-1 inhibition was based on the protocol of the "Human β-Secretase (BACE-1) Inhibitor Screening Assay" kit (BioVision), which is based on the fluorometric method (Tok et al., 2022).

4.3 | Molecular docking studies and molecular dynamics studies

The in silico docking procedure was applied to understand potential interactions, which point out to us what's the relation between ligands and acetylcholinesterase and ß-secretase enzymes (PDBID: 4EY7 and 2ZJM, respectively). Herewith, it helps us to understand the behavior of how active compounds act in the active region of the enzyme. Considering in vitro enzyme tests, compounds **3b**, **3c**, **3f**, and **3j** were docked into AChE active pocket and BACE-1 binding cavity using structure-based in silico docking procedure. After docking studies, to understand environmental effects regarding time on the stability and behavior of ligand-protein complex, the most active compound on both enzymes, **3c** and **3j**, was used as a model for its analogs. The MDS method for at least 50 ns simulation time was applied the same as performed previously by our team (AL-Sharabi et al., 2023; Evren, 2022b; Naji et al., 2023).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information material of this article.

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